Caenorhabditis elegans NPR-1–mediated behaviors are suppressed in the presence of mucoid bacteria

Kirthi C. Reddy[a], Ryan C. Hunter[b], Nikhil Bhatla[a,c], Dianne K. Newman[b,d], and Dennis H. Kim[a,1]

[a]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; [b]Howard Hughes Medical Institute and Division of Biological Sciences, California Institute of Technology, Pasadena, CA 91125; [c]Graduate Program in Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; and [d]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125

Edited* by Frederick M. Ausubel, Harvard Medical School and Massachusetts General Hospital, Boston, MA, and approved June 30, 2011 (received for review May 23, 2011)

Caenorhabditis elegans **exhibits a diverse range of behaviors in response to bacteria. The presence of bacterial food influences *C. elegans* aerotaxis, aggregation, locomotion, and pathogen avoidance behaviors through the activity of the NPR-1 neuropeptide receptor. Here, we show that mucoid strains of bacteria that produce an exopolysaccharide matrix do not induce NPR-1–dependent behaviors. In the presence of mucoid strains of bacteria, the *C. elegans* laboratory wild-type (WT) strain N2 exhibits behaviors characteristic of wild isolates and mutants with reduced NPR-1 activity. Specifically, N2 exhibits lawn bordering and roaming behavior on mucoid nonpathogenic bacteria and loss of pathogen avoidance on mucoid *Pseudomonas aeruginosa*. Alginate biosynthesis by laboratory and clinical isolates of mucoid *P. aeruginosa* is necessary and sufficient to attenuate NPR-1–mediated behavior and it suppresses *C. elegans* pathogen avoidance behavior. Our data suggest that the specific interaction with nonmucoid bacteria induces NPR-1–dependent behaviors of *C. elegans*. These observations provide an example of how exopolysaccharide matrix biosynthesis by a community of bacteria may inhibit specific host responses to microbes.**

Immune recognition of microbial pathogens is critical for the survival of multicellular organisms, and microbes have evolved strategies to subvert immune recognition and effector mechanisms. *Caenorhabditis elegans* responds to pathogenic microbes with conserved innate immune signaling responses (1–3), but detection of microbes by the sensory nervous system also has important roles in homeostasis (4–6) and defense (7). For *C. elegans*, bacterial food in soil or in decaying organic matter serves as an essential source of nutrients (8), but also may cause lethal infection. In the laboratory, *C. elegans* is commonly cultivated on live bacterial food sources that are nonpathogenic (9). In response to exposure to pathogenic bacteria, *C. elegans* exhibits aversive learning behavior (7). For such behavioral responses to pathogens, in which *C. elegans* discriminates between pathogenic and nonpathogenic bacteria, microbe-specific signals might be anticipated, and at least two such bacterial products have been implicated in the induction of avoidance behavior (10, 11).

A set of related *C. elegans* behaviors induced by the presence of bacteria is dependent on the activity of NPR-1, a neuropeptide receptor (12). The laboratory wild-type (WT) strain N2 has the 215V allele of *npr-1*, which has increased NPR-1 activity relative to the 215F allele found in wild isolates of *C. elegans*, such as CB4856 (12, 13). NPR-1 influences the locomotion of *C. elegans* in a food-dependent manner. The wild-type N2 strain strongly reduces its speed of locomotion and increases its rate of turning when bacterial food is present. Worms with reduced NPR-1 activity such as the CB4856 strain move at a high speed with a low turning rate both in the presence and absence of bacterial food (12, 14).

In addition, in the absence of bacterial food, N2 and CB4856 strains, as well as *npr-1* loss-of-function (*lf*) mutants (isolated in the N2 background), prefer an oxygen concentration of about 8–10% in a flow cell chamber using an oxygen gradient (15). However, the presence of bacteria modifies the aerotaxis behavior of N2. Instead of migration toward 8–10% oxygen, N2 distributes itself evenly across an oxygen gradient. In contrast, bacteria have no effect on the aerotaxis behavior of CB4856 or *npr-1* mutants, which continue to migrate toward 8–10% oxygen in the presence of bacteria (15, 16).

C. elegans is propagated in the laboratory on agar plates that have been seeded with bacteria, commonly the *Escherichia coli* strain OP50 (9). The resulting bacterial lawn influences *C. elegans* aerotaxis behavior in at least two regards. First, the lawn of metabolically active bacteria generates a radial oxygen gradient, such that at the rim of the lawn where bacterial density is highest, the oxygen concentration is lowest (15). Second, the presence of bacteria alters *C. elegans* aerotaxis behavior in the aforementioned NPR-1–dependent manner (15, 16). Therefore, *npr-1(lf)* mutants or wild isolates with low NPR-1 activity such as CB4856 exhibit bordering behavior where the worms prefer the edge of the bacterial lawn, whereas wild-type N2 does not exhibit this bordering behavior (12, 15, 16) (Fig. S1). That the bordering behavior of *npr-1(lf)* mutants is a consequence of hyperoxia avoidance is most readily apparent from two experiments that eliminate the hyperoxia avoidance drive. First, lowering the oxygen concentration to 10% leads to dispersal of the bordering behavior of *npr-1(lf)* mutants (15, 16). Second, a mutation in *gcy-35*, encoding a guanylate cyclase homolog that functions as a sensor for molecular oxygen and is required for aerotaxis, suppresses the bordering behavior caused by *npr-1(lf)* mutants (14–16). The effect of NPR-1 activity in the presence of bacterial food confers N2 with a nonbordering phenotype.

The NPR-1–dependent influence of bacteria on the aerotaxis behavior of *C. elegans* N2 facilitates avoidance of pathogenic bacteria (17). In the presence of a lawn of pathogenic *Pseudomonas aeruginosa* PA14, *C. elegans* N2 exhibits aversive learning behavior that results in animals leaving the lawn (7, 11, 18, 19). In contrast, *npr-1(lf)* mutants and strains carrying the 215F allele of *npr-1* do not leave the lawn of *P. aeruginosa* because of an overriding hyperoxia avoidance (Fig. S1) (17). This NPR-1–dependent behavioral difference in the presence of pathogenic bacteria underlies the difference in susceptibility between N2 and *npr-1* mutants (17).

These observations suggest that the integration of oxygen sensing and bacteria sensing may aid *C. elegans* in striking a balance between the need for food and the risk of infection (17). Recent studies suggest that the 215V allele of *npr-1* likely emerged through a mutation during the laboratory domestication of the Bristol N2 strain (20, 21). Thus, the NPR-1–de-

Author contributions: K.C.R. and D.H.K. designed research; K.C.R. and R.C.H. performed research; R.C.H., N.B., and D.K.N. contributed new reagents/analytic tools; K.C.R., R.C.H., N.B., D.K.N., and D.H.K. analyzed data; and K.C.R. and D.H.K. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: dhkim@mit.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1108265108/-/DCSupplemental.

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pendent behaviors observed in the *C. elegans* N2 strain may not be observed to the same degree in wild populations of *C. elegans*, all of which have been found to carry the 215F allele of *npr-1*, which has reduced, albeit nonnull, activity. Nevertheless, the NPR-1–dependent behaviors of the N2 strain represent a robust, experimentally tractable set of responses to bacteria in which to probe the molecular interactions between a simple host and microbe. In the present study, we sought to define the bacterial determinants of NPR-1–dependent behavior. Here, we show that exopolysaccharide matrix production by mucoid strains of bacteria is necessary and sufficient to attenuate *C. elegans* NPR-1–dependent behaviors that are induced by nonmucoid bacteria. These data provide an example of how host organism responses to microbes may be impaired by the bacterial synthesis of an exopolysaccharide matrix. Moreover, our data also suggest that bacterial cues, and not the presence of adequate nutrition, serve as the signal to induce NPR-1–dependent behavior.

Results

Reversal of *C. elegans* NPR-1–Dependent Behaviors in the Presence of Mucoid Strains of Nonpathogenic Bacteria. We hypothesized that specific bacterial signals might be involved in the food-dependent regulation of aerotaxis behavior, and thus we screened diverse species of bacteria to identify strains on which the *C. elegans* wild-type N2 strain would exhibit the bordering behavior observed in *npr-1(lf)* mutants and CB4856 (Fig. S1). *Sinorhizobium meliloti* is a Gram-negative bacterium, which is essential to bacteria–plant symbiosis and nitrogen fixation (22), and has been previously shown to be a relatively nonpathogenic food source, comparable to *E. coli* OP50 in terms of *C. elegans* survival (23). We observed that *C. elegans* N2 exhibited bordering behavior on the lawn of *S. meliloti* (Fig. 1*A*), similar to the bordering behavior exhibited by the *npr-1* mutant and CB4856 on *E. coli* OP50 (Fig. S1). Equivalent bordering behavior was observed for the *npr-1* mutant (Fig. 1*B*).

The lawn bordering behavior of *npr-1* mutants is due to hyperoxia avoidance (15, 16) and thus can be suppressed by a mutation in *gcy-35*, which encodes a guanylate cyclase that functions as a sensor of molecular oxygen (14–16). To confirm that the bordering behavior of N2 on *S. meliloti* was also due to hyperoxia avoidance, we examined the behavior of the *gcy-35* mutant. We found that the *gcy-35* mutant did not exhibit bordering behavior on *S. meliloti* (Fig. 1*B*). In addition, incubation of plates containing N2 on *S. meliloti* at a reduced oxygen concentration of 10% (which abrogates the hyperoxia avoidance drive to migrate to the border of the bacterial lawn) led to loss of the bordering phenotype (Fig. 1*A*).

A defining feature of the *S. meliloti* lawn is its mucoid state due to the production of exopolysaccharides (22). We hypothesized that this mucoid phenotype, not necessarily a *Sinorhizobium* species-specific determinant, was responsible for the bordering behavior of N2. The nonbordering behavior of N2 on *E. coli* strains that have served as bacterial food in the laboratory cultivation of *C. elegans* has long been observed, and thus we sought to examine the behavior of N2 on a mucoid strain of *E. coli*. We observed that on the mucoid *E. coli* strain American Type Culture Collection (ATCC) 35353, N2 also exhibited the bordering phenotype that was observed on *S. meliloti* (Fig. 1*A*).

In addition, we found that the NPR-1–dependent influence of bacteria on the locomotion of N2 *C. elegans* is suppressed by mucoid bacteria. In the presence of nonmucoid *E. coli* OP50, N2 worms move at a low speed (12). In contrast, on a lawn of mucoid nonpathogenic bacteria, N2 moved twice as quickly, a behavior similar to that exhibited by the CB4856 strain. The CB4856 strain, which has reduced NPR-1 function, moved at rapid speed on both mucoid and nonmucoid bacteria (Fig. 1*C*).

If NPR-1–dependent behavior is induced by the presence of nutrients in bacterial food, then mucoid bacteria might not elicit

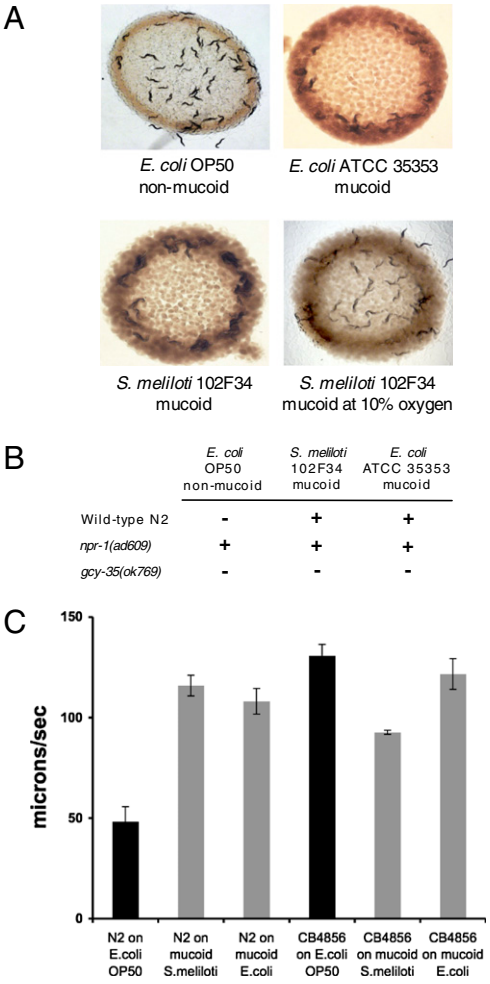

Fig. 1. Reversal of NPR-1–dependent behaviors on mucoid strains of nonpathogenic bacteria. (*A*) Approximately 50 N2 L4 stage worms were cultured on plates and imaged after 24 h at 22 °C on bacterial lawns of the standard laboratory food source nonmucoid *E. coli* strain OP50, mucoid *S. meliloti* strain 102F34, and mucoid *E. coli* strain ATCC 35353. (*B*) Summary of bordering results obtained with indicated strains under conditions as in *A*. +, greater than 80% of the worms were on the border of the lawn; –, fewer than 40% of the worms were on the border of the lawn. (*C*) Average speed of movement of N2 and CB4856 worms on nonpathogenic bacterial lawns of the indicated strains. Black bars indicate nonmucoid strains; gray bars indicate mucoid strains. Three plates were analyzed for each data point, with at least 25 animals scored for 20 min of recording on each plate. Error bars represent the SEM.

NPR-1–dependent responses if mucoid bacteria could not be adequately used as a food source. Indeed, prior studies have shown that bacterial species can vary widely in their ability to be used as food by *C. elegans* (24). To determine whether mucoid *E. coli* and *S. meliloti* are adequate nutrition sources for *C. elegans*, we assayed the egg-laying rate and timing of development of N2 worms on these nonpathogenic strains of mucoid bacteria. We found that *C. elegans* growth and reproduction on these mucoid strains are comparable to that observed for animals propagated on *E. coli* OP50. The number of eggs laid per hour by N2 on *S. meliloti* (5.5 ± 0.85) and mucoid *E. coli* (4.6 ± 1.43) was similar to that observed for N2 on *E. coli* OP50 (5.6 ± 1.35). In addition, we found no change in the time needed for N2 worms to develop from eggs to the L4 larval stage (~48 h at 20 °C for all three bacterial strains). Thus, nutritional differences between mucoid and nonmucoid strains do not account for the differential NPR-1–dependent behavior observed.

Mucoid Strains of *P. aeruginosa* Do Not Elicit Behavioral Avoidance by *C. elegans* N2. We proceeded to examine how *C. elegans* lawn avoidance behavior is affected by mucoid strains of the pathogenic bacterium, *P. aeruginosa*, for which the mucoid phenotype has been well characterized due to its clinical significance (25). Mutation of the *P. aeruginosa* PA14 *mucD* gene, which encodes a homolog of the *E. coli* gene *degP*, results in a mucoid phenotype due to the overproduction of the exopolysaccharide alginate (26). In contrast to the avoidance behavior of N2 on nonmucoid strains of *P. aeruginosa* PA14, N2 did not avoid the PA14 *mucD* mutant lawn of mucoid *P. aeruginosa* (Fig. 2 *A* and *B*). Loss of avoidance behavior of *C. elegans* N2 was also observed on four out of four additional mucoid pathogenic strains tested, including three strains of *P. aeruginosa* and one mucoid strain of *Burkholderia cepacia* (Fig. 2*B*).

The loss of *C. elegans* behavioral avoidance of mucoid *P. aeruginosa* could be a consequence of the lack of production in mucoid bacteria of a bacterial signal that induces avoidance behavior, such as compounds produced by pathogenic bacteria that have been implicated in *C. elegans* avoidance behavior (10, 11). However, our observations of N2 exhibiting bordering behavior on nonpathogenic mucoid bacteria (Fig. 1 *A* and *B*) indicated that the lack of avoidance of pathogenic mucoid *P. aeruginosa* by N2 might be a consequence of hyperoxia avoidance by N2—just as with nonpathogenic mucoid bacteria, pathogenic mucoid bacteria might impair NPR-1–dependent activity that modifies aerotaxis behavior.

To distinguish between these possibilities, we used the *gcy-35* mutant, in which aerotaxis behavior is attenuated (15, 27). If the lack of avoidance were due to diminished production of a bacterial signal or attenuated virulence of mucoid strains, then we would not anticipate a mutation in *gcy-35*, which principally influences behavioral responses to changes in environmental oxygen concentration, to affect the loss of behavioral avoidance. Alternatively, if the lack of avoidance of mucoid *P. aeruginosa* were due to hyperoxia avoidance in the setting of attenuated NPR-1–dependent behavior, then we would anticipate that a mutation in *gcy-35* would restore pathogen avoidance behavior in the presence of mucoid *P. aeruginosa*. The latter is what we observed for the *gcy-35* mutant on the mucoid *P. aeruginosa* PA14 *mucD* lawn (Fig. 3 *A* and *B*). Corroborating these observations, we found that lowering the oxygen concentration to 10%, thus eliminating the tendency of hyperoxia avoidance to drive *C. elegans* into the bacterial lawn, also restored pathogen avoidance behavior (Fig. 3*B*).

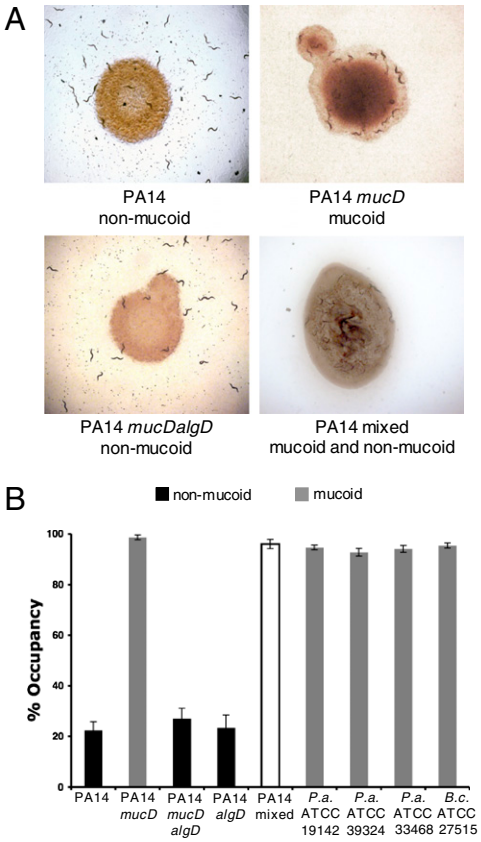

Fig. 2. *C. elegans* N2 does not avoid mucoid *P. aeruginosa*. (*A*) Approximately 30 L4 larval stage *C. elegans* N2 were placed on lawns of *P. aeruginosa* strains PA14, PA14 *mucD*, PA14 *mucDalgD*, and a mixed lawn of PA14 and PA14 *mucD* and imaged after 24 h at 25 °C. (*B*) Lawn occupancy of N2 worms on mucoid and nonmucoid pathogenic bacterial strains ($n > 300$ worms for each bacterial strain). Small lawns of bacterial strains were cultured on 3-cm plates, and L4 worms were placed in the center of each lawn. The number of animals on each lawn was counted after 24 h at 25 °C. Strains PA14, PA14 *mucDalgD*, and PA14 *algD* are nonmucoid (black bars); strains PA14 *mucD*, *P. aeruginosa* ATCC 19142, *P. aeruginosa* ATCC 39324, *P. aeruginosa* ATCC 33468, and *B. cepacia* ATCC 27515 are mucoid (gray bars). Error bars represent the SEM of at least 15 independent plates.

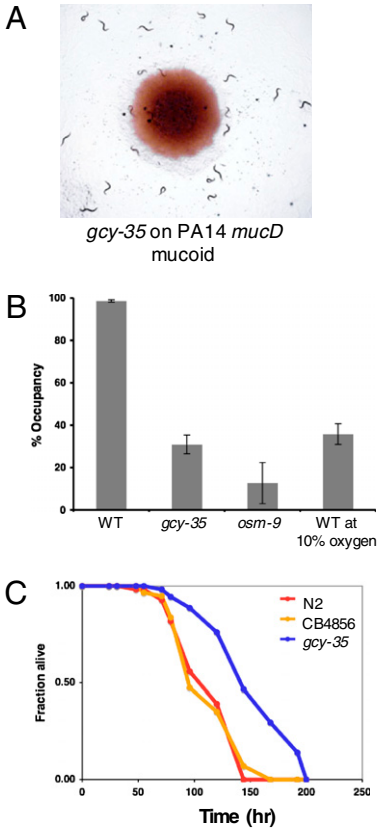

Fig. 3. Inhibition of aerotaxis behavior modulates survival on mucoid *P. aeruginosa*. (*A*) Photograph of loss-of-function mutant *gcy-35(ok769)* worms cultured on a plate with the mucoid *P. aeruginosa* strain PA14 *mucD*. Approximately 30 L4 worms were placed on the lawn and photographed after 24 h at 25 °C. (*B*) Lawn occupancy of indicated *C. elegans* strains on mucoid PA14 *mucD*. (*C*) Survival curve of L4 stage N2, CB4856, and *gcy-35(ok769)* mutant worms on mucoid *P. aeruginosa* strain PA14 *mucD*. The assay was carried out with lawn conditions consisting of a small spot of bacteria in the center of the plate as previously described (19). At least 60 worms for each genotype were assayed in two independent trials. The differences in susceptibility between N2 and *gcy-35(ok769)* and between CB4856 and *gcy-35(ok769)* are significant ($P < 0.0001$ for each comparison).

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These data suggest that mucoid bacteria do not induce NPR-1–dependent modification hyperoxia avoidance that is normally observed for *C. elegans* N2 on nonmucoid bacteria. Diminished activity of NPR-1, leading to hyperoxia avoidance in the presence of bacteria, can be suppressed not only by mutations in *gcy-35*, but also in *ocr-2* and *osm-9*, which encode subunits of a transient receptor potential vanilloid (TRPV) channel that functions in ASH neurons to inhibit hyperoxia avoidance (16). We observed that an *osm-9* mutant exhibited avoidance of mucoid *P. aeruginosa* PA14 *mucD* (Fig. 3*B*), consistent with the apparent lack of induction of NPR-1–mediated behavior by mucoid *P. aeruginosa*. Expression of *gcy-35* in the URX, AQR, and PQR neurons and expression of *osm-9* in the ASH neurons has previously been shown to restore the NPR-1–dependent oxygen sensitivity of these mutants (15, 16), and we found that this expression can also rescue the mucoid pathogen avoidance behavior of these mutants (Fig. S2).

Alginate Biosynthesis by Mucoid *P. aeruginosa* Attenuates NPR-1–Dependent Behavioral Avoidance. Characterization of mucoid *P. aeruginosa* strains have revealed that in addition to exopolysaccharide biosynthesis, which confers the mucoid appearance of the isolates, mutations causing the mucoid phenotype can also affect a wide range of additional phenotypes including mechanisms of virulence (26). We sought to determine whether the lack of NPR-1–dependent modification of *C. elegans* aerotaxis behavior is specifically a consequence of exopolysaccharide biosynthesis or a different property of mucoid bacteria. To decouple alginate hyperproduction in the PA14 *mucD* mutant from the pleiotropic effects of the *mucD* mutation, we investigated the avoidance behavior of *C. elegans* in the presence of the PA14 *mucD* mutant that also carries a mutation in the *algD* gene, which knocks out alginate biosynthesis (26). The effect of the *algD* mutation is to revert the mucoid state of the bacterial lawn, but other pleiotropies are not markedly altered (26). We observed that *C. elegans* avoided the PA14 *mucDalgD* mutant lawn (Fig. 2 *A* and *B*). These data suggest that alginate biosynthesis, not effects of the *mucD* mutation independent of alginate production, causes the impaired *C. elegans* avoidance of PA14 *mucD*. These experiments, comparing two strains differing only in the alginate biosynthetic enzyme *algD*, further suggest that mucoid exopolysaccharide matrix production blocks the induction of NPR-1–mediated behavioral responses to bacteria.

We further investigated whether the presence of alginate synthesized by mucoid strains could block the avoidance responses induced by wild-type PA14. We generated a mixed *P. aeruginosa* lawn consisting of heat-killed PA14 *mucD* and live WT PA14. We observed that *C. elegans* N2 did not avoid this mixed lawn (Figs. 2 *A* and *B*), suggesting that the production of alginate is sufficient to attenuate *C. elegans* NPR-1–dependent behavior.

The thick consistency of the mucoid *P. aeruginosa* lawn led us to consider whether dramatic differences in oxygen tension within mucoid and nonmucoid lawns might contribute to the observed behavioral differences. We measured oxygen concentrations across the depth of the *P. aeruginosa* PA14 WT and *mucD* lawns at the center and at the edge of the bacterial lawns. We observed similar profiles for WT (Fig. 4*A*) and *P. aeruginosa* PA14 *mucD* (Fig. 4*B*) lawns. In comparison with a lawn of *E. coli* OP50, oxygen concentrations within the PA14 lawn were uniformly lower, more closely approximating the preferred oxygen concentrations of *C. elegans* (15, 16). *P. aeruginosa* can exhibit high levels of oxidative metabolism (28), and thus increased oxygen consumption likely accounts for these differences. In addition, the lower oxygen concentration of *P. aeruginosa* lawns explains why we do not observe bordering behavior of *C. elegans* in the *P. aeruginosa* lawns. In the *P. aeruginosa* lawns, the center of the lawn is closer to the preferred oxygen concentration of *C. elegans*, and thus there is not as steep a gradient attracting animals to the border. Under the conditions of these experiments, we did not observe substantial differences between the WT and *P. aeruginosa* PA14 *mucD* oxygen profiles that could account for behavioral differences of *C. elegans*. Differences in oxygen concentration between the center and edge of *P. aeruginosa* lawns are not likely to be simply a consequence of different densities of bacteria, but rather, differences in bacterial oxidative metabolism that are influenced by the position of bacteria within the lawn. Studies of bacterial metabolism within colony models of biofilms suggest that differences in oxidative metabolism may exist at small spatial scales (29).

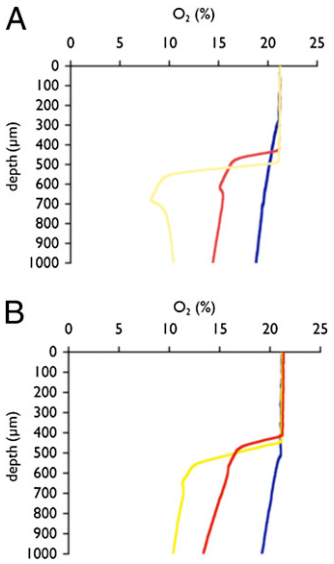

Fig. 4. Oxygen concentration measurements of WT and *mucD P. aeruginosa* PA14 lawns. Representative profiles of oxygen concentration as a function of depth throughout (*A*) PA14 and (*B*) *mucD* colonies. Duplicate measurements were taken at the border (yellow) and center (red) of each colony for two independent colonies. Agar (blue) alone was measured as a control. Measurements up to ~400 μm reflect ambient oxygen concentrations before the electrode entering the colony.

***C. elegans* Mutants Defective in Oxygen Sensation Have Enhanced Survival on Mucoid *P. aeruginosa*.** The previously reported characterization of mucoid *P. aeruginosa* PA14 *mucD* included the killing of *C. elegans* N2 (26). We have shown that a marked difference in susceptibility between *C. elegans* N2 wild type and strains with diminished NPR-1 activity, such as *npr-1(lf)* mutants and wild strains such as CB4856, results from the effects of NPR-1 on *C. elegans* behavioral avoidance (17). Because mucoid strains do not elicit NPR-1–mediated behavior, we anticipated that N2 and CB4856 strains should exhibit the same susceptibility to killing by a lawn of mucoid *P. aeruginosa*. Consistent with this expectation, we observed equivalent survival for *C. elegans* N2 and CB4856 strains on mucoid *P. aeruginosa* PA14 *mucD* (Fig. 3*C*). These data further corroborate the behavioral basis for NPR-1–mediated differences in susceptibility of *C. elegans* strains to pathogenic *P. aeruginosa* (17). Furthermore, our studies of behavioral avoidance suggest that disabling aerotaxis behavior should restore avoidance of mucoid *P. aeruginosa* PA14 and confer a survival benefit. Indeed, we observed that the *gcy-35* mutant, which does not exhibit hyperoxia avoidance and hence does exhibit avoidance of the lawn of pathogenic *P. aeruginosa* (Fig. 3 *A* and *B*), had enhanced survival compared with wild-type N2 and CB4856 strains (Fig. 3*C*).

Discussion

C. elegans behavior in and around a lawn of bacteria represents the integrated output of responses to multiple cues, including an attraction to food, aversive learning in response to pathogenic bacteria, and oxygen concentration (Fig. 5). NPR-1 activity strongly modulates roaming behavior, alters hyperoxia avoidance behavior in the presence of bacteria, and facilitates the avoidance of pathogenic bacteria (14–17). We observed that the presence of mucoid bacteria, both nonpathogenic and pathogenic, strikingly mimicked the effects of diminished NPR-1 activity. Our data on the behavior, growth, and reproduction of N2 animals on mucoid bacteria establish that nutrition alone is not the environmental cue for NPR-1–mediated behaviors in *C. elegans*. There may be survival benefits to integrating foraging behavior and responses to oxygen concentration with the detection of bacteria (12, 16, 17).

Exopolysaccharide biosynthesis is an integral part of biofilm formation in diverse bacterial species (30). The emergence of mucoid forms of *P. aeruginosa* in chronic pulmonary infections in patients with cystic fibrosis is associated with a poor prognosis (25). Mucoid strains of *P. aeruginosa* appear to have increased resistance to antibiotic therapy and innate and adaptive immune responses, but the mechanisms are not well understood. In vitro studies of murine leukocyte killing have indicated impaired survival of *P. aeruginosa* mutants defective in alginate synthesis relative to wild-type *P. aeruginosa* (including PA14) and mucoid *P. aeruginosa* strains (31). These data were suggestive of impaired phagocyte-mediated killing stimulated by IFN-γ and do not indicate increased resistance of mucoid strains relative to nonmucoid strains, but suggest a functional role for alginate biosynthesis in the ability of *P. aeruginosa* to survive phagocyte-mediated killing. A study of biofilm mutants of *Staphylococcus epidermidis* in *C. elegans* was suggestive of an increased protective benefit to bacteria expressing exopolysaccharide in the *C. elegans* intestine (32). These data were interpreted in terms of exopolysaccharide biosynthesis conferring increased protection against antimicrobial peptide synthesis. Whether the presence or absence of exopolysaccharide in *S. epidermidis* influenced behavioral avoidance responses of *C. elegans* strains used in that study will require further investigation.

Our data suggest that the exopolysaccharide matrix of mucoid nonpathogenic bacteria and pathogenic *P. aeruginosa* impairs a specific *C. elegans* behavioral response to the presence of bacteria. These observations suggest that the masking of host recognition mechanisms by the alginate matrix may underlie the increased resistance of mucoid strains of pathogenic bacteria to host killing mechanisms and the corresponding ability of mucoid *P. aeruginosa* strains to persist in chronic infections. We suggest that just as the production of a polysaccharide capsule represents an important virulence determinant for planktonic bacterial pathogens in the evasion of immune recognition and defense, the biosynthesis of an exopolysaccharide matrix may represent an ancient mechanism by which a community of bacteria evades predation and/or host defense mechanisms.

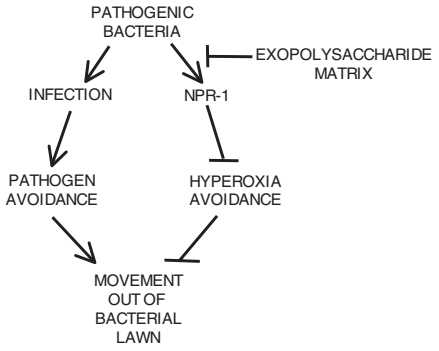

Fig. 5. Bacteria influence *C. elegans* behavior through differential modulation of hyperoxia avoidance and pathogen avoidance responses. The NPR-1–dependent regulation of oxygen sensation behavior by bacteria is impaired by mucoid exopolysaccharide biosynthesis, which can modulate the balance between oxygen sensing and pathogen avoidance behaviors of *C. elegans*.

Materials and Methods

Strains. *C. elegans* was cultured on the bacterial strain OP50 as described (9). N2 (Bristol), CX6448 *gcy-35(ok769)*, and CX10 *osm-9(ky10)* were obtained from the *Caenorhabditis* Genetics Center. CX7265 *osm-9(ky10); yzEx53* and CX6718 *gcy-35(ok769); kyEx737* were provided by C. Bargmann (Rockefeller University, New York, NY). *P. aeruginosa* PA14, PA14 *mucD*, PA14 *algD*, and PA14 *mucDalgD* were provided by F. Ausubel (Harvard Medical School, Massachusetts General Hospital, Boston, MA). *P. aeruginosa* strains ATCC 19142, 33468, 39324, *B. cepacia* strain ATCC 27515, and *E. coli* strain ATCC 35353 were obtained from the ATCC. *S. meliloti* 102F34 was provided by G. Walker (Massachusetts Institute of Technology, Cambridge, MA).

Lawn Occupancy Assays. *P. aeruginosa* and *B. cepacia* bacterial lawns were prepared by spotting agar plates (33) with 5 μL of an overnight culture and incubating the plate at 37 °C for 24 h and then at room temperature (~22 °C) for 24 h before transferring the worms to the plates for the assay. The mixed lawn was generated by spotting agar plates with 5 μL of an overnight culture of PA14 *mucD* and incubating the plate at 37 °C for 24 h, followed by an incubation at 65° for 4 h to kill the mucoid lawn. A total of 5 μL of an overnight culture of WT PA14 was then seeded on top of the killed mucoid lawn and incubated at 37 °C for 24 h.

Approximately 30 *C. elegans* L4 larvae were placed onto the indicated bacteria for each plate, and the number of worms in and out of the lawn were counted and imaged after 24 h at 25 °C. For experiments carried out at 10% oxygen concentration, a mass flow controller (Sierra Instruments) was used to regulate gas flow into an acrylic chamber maintained at room temperature (~22 °C).

Lawn Bordering Assays. *E. coli* OP50, *E. coli* ATCC 35353, and *S. meliloti* 102F34 bacterial lawns were prepared by spotting standard NGM agar plates with 5 μL of an overnight culture (*E. coli* grown at 37 °C, *S. meliloti* grown at 30 °C) and incubating the plate at room temperature (~22 °C) for 48 h before the assay. Approximately 50 *C. elegans* L4 larvae were placed onto the indicated bacteria, and number of worms on the border was scored after 24 h at 22 °C. Five plates were scored for each condition.

Growth and Reproduction Assays. Bacterial lawns were grown as described for the lawn bordering assays. Synchronized gravid adult N2 worms (n = 20 for each condition) were individually placed onto the indicated bacteria and allowed to lay eggs at 20 °C for 1 h. The number of eggs present on the plates was then counted. The plates were then incubated at 20 °C and scored for the time needed for development of these eggs to the L4 larval stage.

PA14 Pathogenesis Assays. Plates were prepared as described previously (33). A small spot of PA14 in the center of the plate ("small lawn") was prepared by dropping 5 μL of an overnight culture without spreading.

Oxygen Profiling. Lawns were prepared as described above for occupancy assays, and measurements were taken after the seeded plates had been incubated at 37 °C for 24 h. Oxygen concentration profiles throughout each lawn were measured with a Clark-type microelectrode (Unisense) containing an oxygen-permeable silicon membrane and an oxygen-reducing cathode polarized against an internal Ag/AgCl anode (34). A potential of −0.8 V was applied between the cathode and counterelectrode, and the current from the cathode (proportional to oxygen partial pressure at the electrode tip) was measured with a PA2000 picoammeter (Unisense). The electrode was calibrated in air (21% oxygen) and a zero measurement was obtained in 100 mL of water bubbled with N2 for 30 min. Oxygen profiles throughout the center of each lawn, the outer rim, and agar alone were obtained by lowering the electrode, stepwise in 2-μm increments, into the colony. Movements were controlled by a motorized micromanipulator stage and motor controller. Data were logged using SensorTrace PRO software.

Locomotion Analysis. Videos of worm locomotion were recorded using a gray-scale camera mounted above a plate of worms, with the plate illuminated by a red LED ring light. Videos were analyzed using custom Matlab software, which will be described in more detail elsewhere. Briefly, the software identifies worms by analyzing the shape of bright objects in each video frame. Shapes that look like worms are labeled as worms and followed through subsequent frames. Worm-like shapes that overlap between frames are considered to be the same worm moving through space. Worm speed is determined by calculating the distance traveled by a worm's centroid between subsequent frames divided by the duration between frames. If a worm collides with another object, the software stops analyzing the worm until it separates from the collision.

ACKNOWLEDGMENTS. We thank F. Ausubel and G. Walker for bacterial strains, and C. Bargmann for *C. elegans* strains. *C. elegans* strains were also provided by the Caenorhabditis Genetics Center, which is supported by the National Institutes of Health (NIH). K.C.R. was supported by Postdoctoral Fellowships from the Jane Coffin Childs Cancer Research Foundation and the Charles A. King Trust Foundation. R.C.H. was supported by a Canadian Cystic Fibrosis Foundation Postdoctoral Fellowship. N.B. was supported by a National Science Foundation Graduate Research Fellowship. D.K.N. is an Investigator of the Howard Hughes Medical Insitute. This work was supported by NIH GM084477, a Burroughs Wellcome Fund Career Award in the Biomedical Sciences, and a Swanson Career Development Award (Massachusetts Institute of Technology) (to D.H.K.).

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